been proposed which includes the step of encapsulating a drug in a liposome and bonding an antibody to surface of the liposome. In the field of cancer treatment, in particular, a number of reports have been made on efficacy of an antibody-bonded liposome in which an antitumor agent is encapsulated (Konno et al., Cancer Res., 47, 4471, 1987; Japanese Patent Unexamined Publication (Kokai) No. 58-134032). Moreover, a method of bonding polyethylene glycol to a liposome has been proposed as a method for solving problems with a liposome, i.e., leakage of encapsulated substances, agglutination of liposomes, capture in reticuloendotherial organs and the like (Japanese Patent Unexamined Publication (Kokai) Nos. 1-249717 and 2-149512; Klibanovet, A.L. et al., FEBS Lett., 268, 235, 1990).

Page 6, last paragraph

Methods for preparing the liposome of the present invention are not particularly limited and any method available to those skilled in the art can be used. Further, a structure of the liposome of the present invention is not particularly limited and the liposome may be in any structure. For example, the liposome may be any of a multilamellar liposome (MLV) obtained by adding an aqueous solution to a thin lipid membrane formed on a glass wall and subjecting the membrane to mechanical shaking; a small unilamellar liposome (SUV) obtained by the sonication method, the ethanol injection method or the French press method; and a large unilamellar liposome (LUV) obtained by the surfactant removing method, the

reversed-phase evaporation method (Liposome, Sunamoto J. et al., Nankodo Co., Ltd., 1988), or the extrusion method in which MLV is extruded through a membrane having a uniform pore size by pressurization (Liposome Technology, 2nd edition, vol.1, 121, 1993). The particle diameter of the liposome is, for example, 300 nm or smaller, preferably, about 30 to 200 nm.

Page 10, second full paragraph

For example, when IgG is used, an F(ab')2 fragment can be obtained by using an enzyme such as pepsin, and then a thiol group, generated in a Fab' fragment which is obtained by reducing the F(ab')2 fragment by using dithiothreitol, can be utilized in the bonding reaction with a liposome (Martin, F.J. et al., Biochemistry, 20, 4229, 1981). Where IgM is used, a thiol group of an Fc region of IgMs obtained by reducing the J chain under a mild condition can be utilized for bonding with a liposome according to the method of Mirror et al. (J. Biol. Chem., 240, 3325, 1965). When the GAH antibody described in Japanese Patent Unexamined Publication (Kokai) No. 5-304987 is used, an F(ab')2 fragment is preferably used. Bonding of a protein such as an antibody added with a thiol group and a liposome containing a maleimide group is achieved by a reaction in a neutral buffer (pH 6.5 to 7.5) for 2 to 16 hours.

P21620.A03

Page 11, second full paragraph

GAH antibody (a monoclonal antibody described in Japanese Patent Unexamined Publication (Kokai) Nos. 4-346918 and 5-304987 reactive to stomach cancer and colon cancer, 3 mg/mL, 14.4 mL) dissolved in 50 mM phosphate buffer (pH 7.5) containing 1 mM EDTA was added with 92.4 μL of 3 mg/mL iminothiolane and reacted at 37°C for 1 hour to introduce thiol groups (Biochemistry, 12, 3266, 1973). The reaction mixture was subjected to gel filtration and the buffer was exchanged with 0.1 M phosphate buffer (pH 6.0) containing 1 mM EDTA. Then, the resulting thiolated antibody (0.21 mg, 1.7 mg/mL) per 1 mg liposomal doxorubicin was reacted with the liposome at 25°C for 1 hour to form bonding of the antibody to the liposome.

Page 13, second paragraph

100 μL of the plasma was added with 1 mL of 0.3 M hydrochloric acid/50% ethanol (hydrochloric ethanol) and heated at 60°C for 10 minutes to extract DXR. The extract was cooled to 4°C and centrifuged at 15,000 rpm for 10 minutes to collect a supernatant. The sample was diluted 4 times with hydrochloric ethanol and its fluorescence was measured at a fluorescence wavelength of 590 nm with an excitation wavelength of 490 nm. A calibration curve was prepared by using DXR diluted with hydrochloric ethanol to known concentrations and used in quantification of DXR in plasma. The recovery of DXR from the